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PII:	S0260-8774(19)30293-6
DOI:	10.1016/j.jfoodeng.2019.07.008
Reference:	JFOE 9661
To appear in:	*Journal of Food Engineering*
Received Date:	25 April 2019
Accepted Date:	09 July 2019

Please cite this article as: Kaiyun Luo, Shutao Liu, Song Miao, Benu Adhikari, Xufeng Wang, Jie Chen, Effects of Transglutaminase Pre-crosslinking on Salt-Induced Gelation of Soy Protein Isolate Emulsion, *Journal of Food Engineering* (2019), doi: 10.1016/j.jfoodeng.2019.07.008

Effects of Transglutaminase Pre-crosslinking on Salt-Induced Gelation of Soy Protein Isolate Emulsion

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13 **TGase Pre-crosslinking Affects Salt-Induced Gelation of SPI Emulsions**

Abstract: The salt-induced gelation behavior of soy protein isolate (SPI) emulsions was markedly influenced by microbial transglutaminase (TGase) pre-crosslinking. Rheological data showed that when SPI emulsions were incubated with TGase at low concentrations (1 and 3 U/g protein) at 50°C for 30 min prior to gelation, no change in storage modulus (G'), but enhanced resistance to deformation of the gels was observed. Extensive crosslinking by TGase (5 U/g protein) resulted in severe decreases in gel firmness and fracture properties (yielding stress and strain), likely due to the impairment of hydrophobic bonds and the formation of coarse networks. The water-holding capacity of the gels was significantly enhanced by increased concentrations of TGase. Interactive force analysis indicated that non-covalent interactions and disulfide bonds are the primary forces involved in $CaSO_4$-induced SPI emulsion gel, but TGase treatment may limit hydrophobic interactions within the gel network. These results are of great potential value for the application of TGase in the food industry.

Keywords: Transglutaminase; Pre-crosslinking; Soy protein isolate; Emulsion gel; Salt

1. Introduction

Human consumption of soy protein (SP) has increased in recent years due to its nutritious benefits to health and function. For example, protein/emulsion gel is one of the most important applications of SP in the food industry, including its use in tofu, soy yogurt, processed meats, and delivery/release systems for bioactive compounds (Abaee, Mohammadian & Jafari, 2017; Nishinari, Fang, Nagano, Guo & Wang, 2018). Generally, the quality of a gelled food is determined by its textural features, including gel strength, water-holding capacity, syneresis property, and microstructure. To obtain food products with improved quality, numerous studies have been undertaken to manipulate the physicochemical properties of proteins by physical, chemical, or enzymatic methods, such as heat treatment (Wang et al., 2018); addition of salts, acids, and other food components (e.g., polysaccharides) (Chen, Zhao, Chassenieux, & Nicolai, 2017; Zhao, Wang, Li, Qin & Chen, 2017); Maillard reaction and enzyme crosslinking (Gan, Cheng & Easa, 2008; Gaspar & de Góes-Favoni, 2015).

Enzyme crosslinking is an effective and green approach to modify protein properties, in which microbial transglutaminase (TGase) is commonly used in the food industry. TGase-induced crosslinking reactions can improve texture, stability, and water-binding capacity without changing the flavor and nutritional quality of food. Recently, TGase has been widely used in the processing of food products (Gharibzahedi et al., 2018). For example, TGase can impart a similar appearance, resembling that of intact muscle, to restructured meat, but with improved mechanical properties and lower losses during cooking (de Góes-Favoni & Bueno, 2014). Addition of TGase increases the gel properties of pork and fish mixtures (Li, Gui, Huang, Feng & Luo, 2018). In the dairy industry, TGase treatment has been successfully used in the manufacture of yogurt to prevent syneresis and

improve gel homogeneity (Abou-Soliman, Sakr & Awad, 2017; Ray & Rosell, 2017). Some researchers are trying to apply TGase as a preliminary treatment in the preparation of protein gels, and the potential capabilities of TGase for modifying food proteins have been elaborated. Adding TGase to raw peanut milk at a concentration of 0.75 mg/g significantly improved the texture of peanut tofu (Guo, Hu, Wang & Liu, 2018). In the acidification process of skim milk, TGase pre-treatment resulted in stiffer gels with greater storage modulus and fracture stress due to the introduction of intermolecular chemical crosslinks (Silva et al., 2018). Moreover, TGase treatment was found to improve water retention in acid milk gels and suppress retort-induced water release and improve the gel quality of glucono-δ-lactone (GDL)-induced tofu (Ercili-Cura et al., 2013; Kwan & Easa, 2003).

TGase can catalyze the acyl-transfer reaction between the γ-carboxyamide groups of glutamine residues and the ε-amino groups of lysine residues in proteins, leading to inter- or intramolecular crosslinking (Romeih & Walker, 2017). As a result, TGase treatment changes protein properties, such as solubility, net charge, and amphiphilic properties, as well as its interactions with other food components, which may further influence the protein functionalities (e.g., gelation, emulsification, and foaming properties) (Ali, Ahmed, Mohamed, Ahmed & Babiker, 2010; Damodaran & Agyare, 2013). To date, numerous studies have investigated the effects of TGase on the functional properties of proteins, such as emulsifying and gelling properties (Chen et al., 2016; Liu, Damodaran & Heinonen, 2019; Mohammad Zadeh, O'Keefe, Kim & Cho, 2018). In addition, gelation of soy protein isolate (SPI) emulsions induced by TGase as a strategy for the delivery and release of bioactive compounds in foods has been well documented in recent years (Tang, Luo, Liu & Chen, 2013; Wang et al., 2018; Yang Liu & Tang, 2013). However, few studies have investigated the use

of TGase as a pre-treatment in food processing and its influence on the characteristics of SPI emulsion gel systems. Understanding of the mechanism of the effect of TGase crosslinking on the properties of SPI emulsions and subsequent gelation behavior would be of great value for food manufacturers in designing gel products with desired textural properties. In such context, the objective of this study was to investigate the effects of TGase pre-crosslinking on the physicochemical, rheological, and microstructural properties of SPI emulsions and emulsion gels induced by salt.

2. Materials and methods

2.1 Materials

SPI was extracted from defatted soybean flour (Taiwan 292) with a protein content of 91.6% (dry weight) according to the method described by Guo, Xiong, Qin, Jian, Huang & Chen (2015). Soy oil was purchased from a local market without any further purification. Nile red and Rhodamin B were obtained from Sigma-Aldrich (St. Louis, MO, USA). Commercial microbial transglutaminase was purchased from Taixing Dongsheng Bio-Tech Co., Ltd (Jiangsu, China). All other chemical reagents were of analytical grade.

2.2 Preparation of SPI emulsion

The stock SPI dispersion (60 mg/mL) was prepared in distilled water and stirred mechanically at room temperature (25 °C) for 2 h, followed by centrifuging at 10,000 g for 10 min to remove insoluble materials. If necessary, the pH of the dispersion was adjusted to pH 7.0 with 0.2 M NaOH or HCl. The SPI dispersion was heated at 95 °C for 15 min in a water bath and then immediately cooled to room temperature.

For the emulsion, the heated SPI dispersion was pre-homogenized with soy oil (5%, v/v) through a disperser (T 18 basic ULTRA-TURRAX®, IKA Corp., Staufen, Germany) at 13,500 rpm for 2 min to obtain a coarse emulsion. The coarse emulsion was further homogenized at 40 MPa for one-pass using a homogenizer (AH-BASIC, ATS Engineering Inc., Canada).

2.3 Pre-treatment of SPI emulsion by TGase

TGase was mixed with the stock SPI emulsions up to enzyme concentrations of 1, 3, and 5 U per gram (U/g) of protein. Then the mixed dispersions were incubated at 50°C for 30 min. The SPI emulsion without enzyme incubated at 50°C for 30 min was defined as control-T, and the SPI emulsion without any treatment (enzyme or incubation) was defined as control-N.

2.4 Preparation of SPI emulsion gel

$CaSO_4$ was used as the coagulant because of its mild reaction rate, which can produce a gel with smooth texture and good flavor. After the incubation process, SPI emulsions pre-treated with TGase were immediately cooled to 25°C and mixed with stock $CaSO_4$ dispersion up to a concentration of 35 mM. The mixtures were then heated to 80°C and allowed to form gels for 30 min in a thermostatic bath. Subsequently, the gels were immediately cooled to 25°C in an ice bath and stored at 4°C.

2.5 Evaluation of emulsion characteristics after enzyme treatment

2.5.1 Oil droplet size

The oil droplet size of the SPI emulsions pretreated by TGase was determined using a laser particle size analyzer (Microtrac S3500, Microtrac, North Largo, FL, USA). Distilled water was used as the dispersant. The relative refractive index of the emulsion was taken as the ratio of the refractive index of soy oil (1.456) to that of water (1.33) (Tang, Chen, & Foegeding, 2011). The

115 volume-average diameter $d_{4,3}$ ($\sum n_i d_i^4 / \sum n_i d_i^3$, where n_i is the number of particles with diameter d_i)
116 was recorded.

117 2.5.2 Confocal laser scanning microscopy (CLSM)

118 The microstructure of the SPI emulsion and emulsion gels was evaluated by confocal laser
119 scanning microscopy (CLSM) (TCS SP8, Leica Microsystems, Heidelberg, Germany), using mixed
120 dyes of rhodamine B (0.1%, for the protein phase) and Nile red (0.1%, for the oil phase), with
121 excitation wavelengths at 552 and 488 nm, respectively. Samples were prepared as follows: a total
122 amount of 80 μL SPI emulsion containing the dyes (5 mL of stock emulsion + 0.05 mL of
123 fluorescence dye) was loaded in single concave slides (Sail Brand, Jinliu Instrument Co., Nanjing,
124 China) and covered with nail oil to prevent water evaporation. The CLSM images were obtained
125 with a 63× magnification lens.

126 **2.6 Dynamic oscillatory measurements**

127 2.6.1 Small amplitude oscillation

128 The dynamic viscoelasticity of the SPI emulsion gels was evaluated using a HAAKE MARS
129 III rheometer (Thermo Fisher Scientific, Karlsruhe, Germany) with a parallel plate (d = 35.002 mm).
130 The gap between the plates was set to 1 mm. After the SPI emulsions were fully mixed with the
131 stock $CaSO_4$ dispersion, the mixtures were immediately loaded between the plates of the rheometer.
132 Low-viscosity silicon oil was used to prevent water evaporation. The emulsions were heated from
133 25°C to 80°C at the rate of 5°C per minute, followed by incubation at this temperature for 30 min
134 before cooling to 25°C at the rate of 5°C per minute. Dynamic oscillations with an amplitude of 1%
135 (within the linear viscoelastic region, LVR) and a frequency of 1 Hz were applied. The storage
136 modulus (G') and loss modulus (G") were recorded.

137 The dynamic viscoelasticity of SPI emulsion gels after the gelling process was characterized in
138 a frequency sweep mode at 25°C, with an angular frequency (ω) from 1~100 rad/s at a constant
139 strain of 1%. Creep-recovery was conducted as described in our previous study (Wang, He, Zeng,
140 Qin, Adhikari & Chen, 2017), a constant stress of 8 Pa was applied, and a four-parameter Burger's
141 model was used to fit the creep data (Steffe, 1996).

142 $$J(t) = \frac{1}{G_0} + \frac{1}{G_1}\left(1 - e^{-\frac{t}{\lambda}}\right) + \frac{t}{\mu_0} \quad (1)$$

143 where J is the creep compliance (1/Pa), G_0 and G_1 represent the instantaneous and retarded elastic
144 modulus (Pa), respectively, λ is the retardation time, and μ_0 is the viscous modulus associated with
145 viscosity flow (Pa · s).

146 2.6.2 Large-scale deformation

147 An oscillatory amplitude sweep was carried out to evaluate the large-scale deformation
148 performance of SPI emulsion gels. The strain was increased from 0.1% up to the fracture point when
149 the stress began to decrease at a frequency of 1 Hz.

150 **2.7 Water-holding capacity**

151 The gel samples were transferred to 50-mL centrifuge tubes and centrifuged at 10,000 *g* for 15
152 min at 4°C. Then the tubes were inverted to drain, and the residue water was carefully removed by
153 filter paper. Water-holding capacity (WHC, %) was defined as the ratio of the weight of water in
154 the pellet to that in the original gel multiplied by 100.

155 **2.8 Dispersibility of the emulsion gels with various solvents**

156 The SPI emulsion gels pretreated under different TGase concentrations were applied to
157 dispersibility evaluation, as described by Tang, Yang, Liu & Chen (2013), with some modifications.
158 The emulsion gels were dispersed in four solvents (2 g sample + 50 mL solvent): D1, distilled water;

D2, a pH 8.0 buffer (0.086 mol/L Tris, 0.09 mol/L glycine, and 4 mmol/L Na_2EDTA); D3, D2 containing 2% sodium dodecyl sulfate (SDS); and D4, D3 containing 1% β-mercaptoethanol (β-ME). The dispersions were then stirred magnetically at room temperature for 1 h. Then the dispersions were centrifuged at 100 *g* for 15 min, and the emulsion layer was subjected to determination of absorbance (A) at 500 nm. The turbidity (T) was calculated as

$$T = (2.303 \times A)/l \quad (2)$$

where l is the optical path length (cm).

2.9 Statistical analysis

All assays were performed in duplicate and repeated at least thrice. An analysis of variance (ANOVA) of the experimental data was performed, and a least significant difference (LSD) test with a confidence interval of 95% was used to compare the means. Simulations of the creep data were modeled using the nonlinear regression feature of SPSS 20.0.

3. Results and discussion

3.1 Oil droplet size

The volume mean diameter ($d_{4,3}$) of the emulsions after TGase treatment is shown in Table 1. Incubation of the SPI emulsions at 50°C without TGase did not change $d_{4,3}$ significantly ($p > 0.05$), showing that SPI emulsions formed with high protein (around 6%) and low oil (5%) content were relatively stable under a mild temperature (50°C). On the other hand, TGase pre-treatment resulted in a progressive increase in the $d_{4,3}$ of oil droplets from 1.03 to 2.22 μm. However, significant increases were observed only when the TGase concentration reached 3 U/g (3 and 5 U/g). The increase in oil droplet size may be due to the formation of high molecular weight biopolymers

induced by TGase and the coalescence of oil droplets. Images of various SPI emulsions after the incubation process are shown in Fig. 1. Larger oil droplets (green colored) were formed when SPI emulsions were treated with TGase at concentrations of 3 and 5 U/g, confirming that incubation with high concentrations of TGase resulted in coalescence of oil droplets in SPI emulsions. This phenomenon was consistent with those reported by Tang et al. (2013), who found that extensive enzyme crosslinking may be associated with impairment of the emulsifying ability of SPI and destabilization of the emulsion. In the present study, TGase pre-crosslinking may have been detrimental to the emulsifying properties of soy protein, resulting in weakened protection of the protein layer absorbed at the surface and thus leading to the coalescence of oil droplets.

3.2 Small deformation rheology of SPI emulsion gels

The gelation behavior of TGase-pretreated SPI emulsions was evaluated by rheological measurements. The storage modulus (G') reflects the elastic (or solid-like) properties of the emulsion gel and is generally used as an indicator of gel firmness (Mao, Roos, Y. H & Miao, 2014). The final G' values of SPI emulsion gels pre-incubated with different concentrations of TGase are shown in Table 2. No obvious differences were observed between SPI emulsion gels incubated with TGase concentrations from 0 (control samples) to 3 U/g, whereas when the TGase concentration was increased to 5 U/g, the firmness of the gel significantly decreased. This observation seems unreasonable, as generally, the formation of covalent crosslinks between protein molecules catalyzed by TGase should enhance gel elasticity (G') and firmness (Spotti, Tarhan, Schaffter, Corvalan & Campanella, 2017). It is noteworthy, however, that incubation with TGase resulted in the coalescence of oil droplets. In emulsion gels, oil droplets usually act as “active fillers”, thus reinforcing the gel network. Larger oil droplets indicate a smaller surface area that is surrounded by

203 protein molecules and aggregates through physicochemical bonds, and even the formation of a
204 heterogeneous network, which may weaken gel strength (Mao, Miao, Yuan & Gao, 2018; Guo, Ye,
205 Lad, Dalgleish & Singh, 2014; Tang et al., 2011). On the other hand, according to Han, Mei, Li, Xu
206 & Wang (2018), the crosslinks formed by enzyme catalysis during incubation may restrict the
207 rearrangement of the protein molecules and therefore the number of possible reaction sites for other
208 intermolecular interactions in the subsequent gelation process (e.g., hydrophobic interactions).
209 Hence, in the present study, the firmness of the SPI emulsions was determined not only by the
210 introduction of covalent crosslinks, but also by the impairment or limitation of noncovalent bonds
211 and gel structure. It can be speculated that excessive TGase crosslinking led to the domination of
212 bond impairment within the gel network and the formation of heterogeneous structures, thus
213 decreasing gel firmness. Similar results have been found in milk gels, in which increase in the
214 content of crosslinked protein (above 25% in raw milk) results in the gels becoming weaker, with
215 higher syneresis (Jaros, Jacob, Otto & Rohm, 2010). Furthermore, it has been reported that
216 polymerization of casein by 80% or more results in gels with the highest stiffness, whereas further
217 crosslinking leads to lower gel strength (Jaros, Schwarzenbolz, Raak, Löbner, Henle & Rohm,
218 2014a; Raak, Schöne, Rohm & Jaros 2019; Rohm, Ullrich, Schmidt, Löbner & Jaros, 2014). Eissa
219 & Khan (2005) demonstrated that after incubation with or without TGase following heat treatment,
220 the subsequent gelation of whey protein induced by GDL resulted in similar storage modulus values.
221 Fig. 2A shows the frequency dependence of G' of various SPI emulsion gels pre-incubated with
222 enzyme at various concentrations. G″ (ω) showed curves similar to but lower in magnitude than
223 those of G′ (G″ < G′) throughout the frequency range from 1 to 100 rad/s (data not shown),
224 suggesting gel-like features (Eissa & Khan, 2005; Zhu, Chen, McClements, Zou & Liu,, 2018). G′

and ω were fitted to the power-law equation ($G' \sim \omega^{n}$), and the n values are presented in Table 2. The results show that for gels without TGase, both moduli showed slight frequency dependence with G' scaling as $\sim \omega^{0.1}$, which is a typical characteristic of a so-called "weak gel" consisting of noncovalent "physical" crosslinks in nature (Hesarinejad, Koocheki & Razavi, 2014). With increasing TGase concentration (1 to 5 U/g), the n values decreased from 0.0909 to 0.0818, suggesting the development of "chemical" covalent bonds within the SPI emulsion gel network, because of the formation of ε-(-γ-Glu) Lys crosslinks induced by TGase.

To further characterize the rheological behavior of SPI emulsion gels, creep-recovery curves of the gels are presented in Fig. 2B. All samples showed typical viscoelastic characteristics, and at the end of the test, irrecoverable strains were observed. Obviously, the emulsion gel pre-incubated with TGase at 5 U/g exhibited the poorest resistance to deformation, indicating low gel rigidity. The elastic and viscous parameters were obtained by modeling the creep data using a simplified Burgers model. As seen in Table 2, the instantaneous elastic modulus (G_0) value did not change significantly when the TGase concentration was increased up to 3 U/g, but it decreased in the gel incubated with TGase at 5 U/g, which was consistent with the variation of G'. The retarded elastic modulus (G_1) is related to the cohesive force of the gels (Wang, Luo, Liu, Adhikari & Chen, 2019); as the TGase concentration increased, more covalent crosslinks were formed, thus increasing the G_1 values. Increasing cohesive forces generally improve gel deformability. The gel incubated with TGase at 5 U/g, however, had high G_1 value but poor deformation resistance. This may be because the gel structure became heterogeneous and was easier to deform under constant applied stress. μ_0 (Pa · s) is the coefficient of viscosity associated with fluid flow (dynamic viscosity). The polymerization of protein molecules induced by TGase pre-treatment reduced water mobility in the protein network

and provided greater flow resistance, and hence, increased the viscosity of the end product (Gaspar & de Góes-Favoni, 2015). In addition, the sharp decrease in the μ_0 value for the TGase-treated (5 U/g) gel may be attributed to the coarse gel network. The relaxation time, λ, is the time required for the delayed strain to reach $1–1/e$ (approximately 63.2%) of the final deformation. The gels with a large λ value reached full deformation slowly (Steffe, 1996).

3.3 Microstructure and fracture properties of SPI emulsion gels

The microstructures of TGase-treated SPI emulsion gels are shown in Fig. 3. The emulsion gels without TGase showed similar gel networks, with fine strands, small oil droplets, and large but uniformly distributed pores. With increasing TGase concentration (~ 3 U/g), the gel structure tended to be much denser and more homogeneous. However, when the TGase concentration reached 5 U/g, the gel became coarse and the oil droplets did not appear to distribute homogeneously within or to firmly bind to the gel network. It can be speculated that excessive intra- and intermolecular crosslinking induced by TGase may lead to the formation of polymers or aggregates that are too large for development of an effective network.

To evaluate the mechanical properties and differences in the structures of TGase-pretreated SPI emulsion gels, large deformation measurements were carried out. Fig. 4 shows the curves of shear stress (τ) as a function of strain (γ); the point at which the stress begins to decrease indicates the fracture point of the gel. TGase treatment significantly increased the fracture stress and strain of the gels. SPI emulsion gels without TGase treatment started to break down at $\tau \sim 1000$ Pa and $\gamma \sim 70\%$. The critical stress and strain for gels from SPI emulsions incubated with TGase at 3 U/g were approximately 1421 Pa and 107%, respectively. The improved fracture behavior (increase in the resistance to deformation) of the gels may be due to the permanent crosslinks formed as a result of

the accumulation of TGase action (Eissa & Khan, 2005). This is consistent with the results reported by Silva et al. (2018), who also found that TGase treatment could improve the yield properties of milk gels. However, the emulsion gel pre-incubated with TGase at 5 U/g displayed decreasing fracture stress and strain, likely because of gel coarseness. Gels with a coarse structure exhibit poor deformability, as the defects in the gel are larger (Renkema, 2004).

3.4 Water-holding capacity

WHC is one of the most important properties of food products. It reflects the ability of the food matrix to effectively immobilize water through capillary effects (Gaspar & de Góes-Favoni, 2015). The WHC data of TGase-pretreated SPI emulsion gels are presented in Table 1. Incubation with TGase markedly enhanced the WHC of SPI emulsion gels. An increase in TGase concentration during incubation from 0 to 5 U/g led to an approximately 20% enhancement in the WHC of the emulsion gels. Previous studies suggested that structure and strength were the primary factors determining the WHC of the gel, as strong and uniform structures tend to "bind" water to a greater extent (Wang et al., 2019). In the present study, with the increase in the TGase concentration during incubation from 0 to 3 U/g, although the firmness (G') of the emulsion gels was comparable, the gel structure became denser and more uniform, with smaller pores or capillaries, thus retaining more water due to stronger capillary forces (Han, Zhang, Fei, Xu & Zhou, 2009). On the other hand, it has been reported that the deamidation of glutamine residues induced by enzyme increases protein hydrophilicity (Renzetti, Bello & Arendt, 2008), and TGase treatment resulted in strong crosslinking of the protein chains, which together likely contributed to the increase in WHC. However, the emulsion gel incubated with TGase at 5 U/g had lower firmness and appeared coarse in structure but exhibited the best WHC. This result seemed unreasonable, as in general, weak gel had low water

retention capacity, indicating that the factors affecting WHC are more complicated. It can be speculated that, in such situation, the properties of the gel network, which consisted of strong covalent crosslinks, as well as changes in the amphiphilicity properties of the protein molecules, may have more effect than overall gel structure and strength on the enhancement of gel WHC.

3.5 Interactive forces involved in gel formation

The interactive forces involved in the formation of the gel network were evaluated by the relative ability of SPI emulsion gels to be dispersed in various solvents (D1–D4). Higher turbidity indicates higher dispersibility of the gel in various solvents and therefore weaker or fewer bonds involved within the gel networks. The differences in turbidity between two adjacent solvents, namely D2–D1, D3–D2, and D4–D3, reflect the contribution of electrostatic forces, noncovalent interactions, and disulfide bonds, respectively. The differences in turbidity between the various emulsion gels in solvent D4 represent enzyme-induced covalent crosslinks contributing to the formation of the gel network (Tang et al., 2013). As shown in Fig. 5 and Table 3, it can be seen that in each solvent, with increasing TGase concentration during incubation, the turbidity progressively and significantly decreased, suggesting the increasing magnitude of interactive forces involved in gel formation. The difference in turbidity between D3 and D2 is much greater than the differences in the other combinations tested, indicating that in the $CaSO_4$-induced gel, noncovalent bonds, particularly hydrophobic interactions, are the most important forces for maintenance of the gel structure. However, for the various emulsion gels, the values of D3–D2 decreased as the TGase concentration increased; this indicates that incubation with TGase decreased the number of noncovalent bonds within the network. This observation confirms the view that enzyme-induced

crosslinking of protein molecules may lead to impairment or limitation of hydrophobic interactions in the formation of SPI emulsion gels, thus reducing gel strength.

4. Conclusions

TGase pre-treatment significantly affected the gelation behavior of SPI emulsions, which was highly dependent on the enzyme concentration during incubation. Pre-treatment of SPI emulsions with low concentrations of TGase (1 and 3 U/g) improved gel structure and resistance to deformation, whereas extensive crosslinking by TGase (5 U/g) resulted in coarse gel structure and a severe decrease in gel firmness as a result of the coalescence of oil droplets and impairment or limitation of hydrophobic interactions. The WHC of the gels increased with increasing concentration of TGase during incubation. Noncovalent interactions and disulfide bonds are the primary forces involved in the formation of $CaSO_4$-induced emulsion gels, but TGase treatment may limit hydrophobic interactions within the gel network. However, because of the complexity of SPI emulsions, the specific effects of TGase crosslinking on protein molecules, such as changes in hydrophobicity, emulsifying properties, and inter- and intramolecular bonds, need to be further quantitatively analyzed in the future. The results of the present study conclusively demonstrate that TGase pretreatment under certain conditions may improve the gelation properties of SPI emulsions, which provide valuable information for the potential application of TGase in the food industry.

Declaration of interest

The authors declare no conflicts of interest.

Acknowledgment

This research was supported by the National Natural Science Foundation of China (NSFC, 31271946), the National High-Tech Research and Development Program of China (863 program,

grant No. 2013AA102200), the National Natural Science Foundation of China (NSFC, 31471583).

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Table 1. Characteristics of SPI emulsion and emulsion gels pre-treated by different concentrations of TGase

Characteristics	TGase concentrations during pre-incubation (U/g)				
	0 (Control-N)	0 (Control-T)	1	3	5
$d_{4,3}$ (μm)	1.03 ± 0.06 ^{c}	1.03 ± 0.05 ^{c}	1.17 ± 0.02 ^{c}	1.55 ± 0.09 ^{b}	2.22 ± 0.09 ^{a}
WHC (%)	67.0 ± 0.7 ^{c}	67.5 ± 0.2 ^{c}	71.0 ± 0.1 ^{b}	75.3 ± 0.9 ^{a}	75.9 ± 0.8 ^{a}

Different lower-case letters (a–c) in the same row indicate significant difference among the values at the 95% confidence level ($p < 0.05$

Table 2. Parameters of rheological properties for SPI emulsion gels pre-treated by different concentrations of TGase

TGase (U/g)	Final G' (Pa)	$G' \propto \omega^{n'}$	Burger's parameters			
		n'	G_0 ($\times 10^3$ Pa)	G_1 ($\times 10^3$ Pa)	μ_0 ($\times 10^6$ Pa s)	λ (s)
0 (Control-N)	1762.3 ± 19.4 [a]	0.1040 ± 0.0005 [a]	1.44 ± 0.04 [a]	3.53 ± 0.07 [c]	2.20 ± 0.11 [c]	32.1 ± 1.3 [ab]
0 (Control-T)	1746.5 ± 4.9 [a]	0.1041 ± 0.0006 [a]	1.46 ± 0.02 [a]	3.58 ± 0.15 [c]	1.98 ± 0.13 [c]	34.5 ± 1.4 [a]
1	1751.0 ± 83.2 [a]	0.0909 ± 0.0002 [b]	1.39 ± 0.07 [a]	3.62 ± 0.07 [c]	2.49 ± 0.11 [b]	31.8 ± 0.7 [b]
3	1764.0 ± 42.4 [a]	0.0871 ± 0.0004 [c]	1.37 ± 0.08 [a]	4.47 ± 0.16 [b]	3.31 ± 0.22 [a]	24.2 ± 0.8 [c]
5	1595.5 ± 50.2 [b]	0.0818 ± 0.0004 [d]	1.12 ± 0.03 [b]	5.92 ± 0.08 [a]	1.24 ± 0.06 [d]	21.3 ± 1.0 [d]

Different lower-case letters (a–d) in the same column indicate significant difference among the values at the 95% confidence level ($p < 0.05$)

Table 3. Difference in the relative turbidity of SPI emulsion gels pre-incubated with different concentrations of TGase between various solvents

TGase	Difference in the relative turbidity between solvents			
(U/g)	D1	D2–D1	D3–D2	D4–D3
0 (Control-N)	0.304 ± 0.010 [a]	0.523 ± 0.003 [a]	11.726 ± 0.018 [a]	3.363 ± 0.020 [a]
0 (Control-T)	0.335 ± 0.052 [a]	0.524 ± 0.003 [a]	12.556 ± 0.091 [a]	3.425 ± 0.027 [a]
1	0.235 ± 0.021 [b]	0.303 ± 0.016 [b]	7.969 ± 0.062 [b]	3.292 ± 0.073 [a]
3	0.094 ± 0.016 [c]	0.212 ± 0.002 [c]	6.247 ± 0.021 [c]	3.242 ± 0.051 [a]
5	0.033 ± 0.002 [d]	0.150 ± 0.014 [d]	5.243 ± 0.081 [d]	3.321 ± 0.066 [a]

Different lower-case letters (a–d) in the same column indicate significant difference among the values at the 95% confidence level ($p < 0.05$)

Declaration of interest

We wish to confirm that there are no known conflicts of interest associated with this publication. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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Figure captions:

Figure 1. CLSM images of SPI emulsions after incubation with different concentrations of TGase.

Figure 2. Frequency dependence of G' (A) and creep-recovery curves (B) for the SPI emulsion gels pre-incubated with different concentrations of TGase

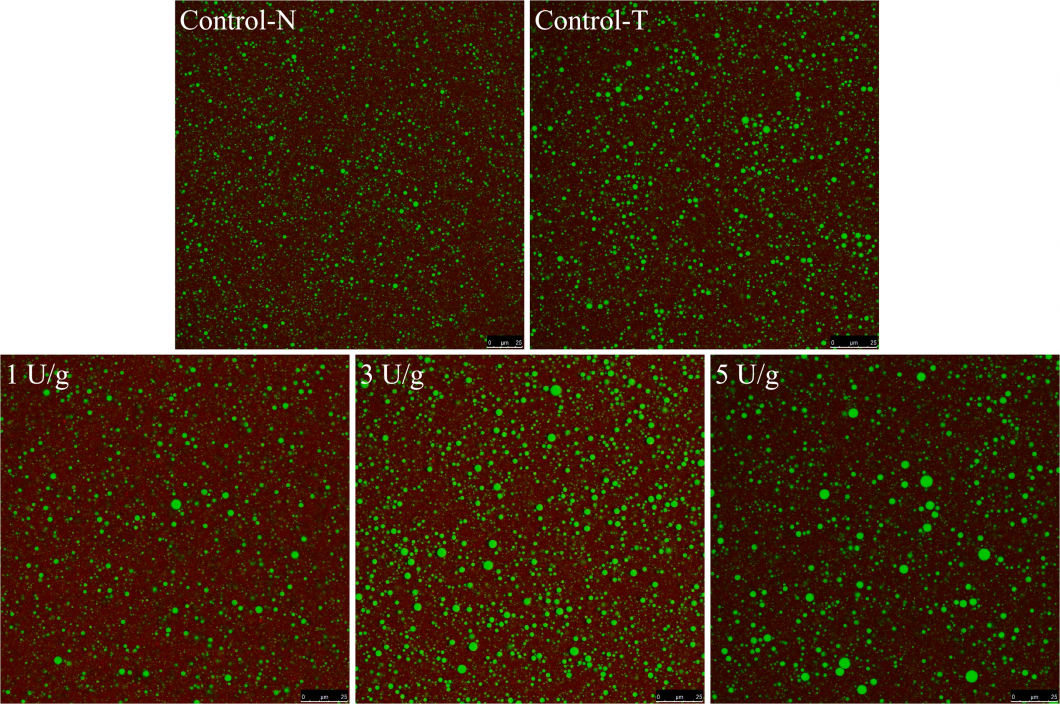
Figure 3. The microstructures of SPI emulsion gels pre-incubated with different concentrations of TGase

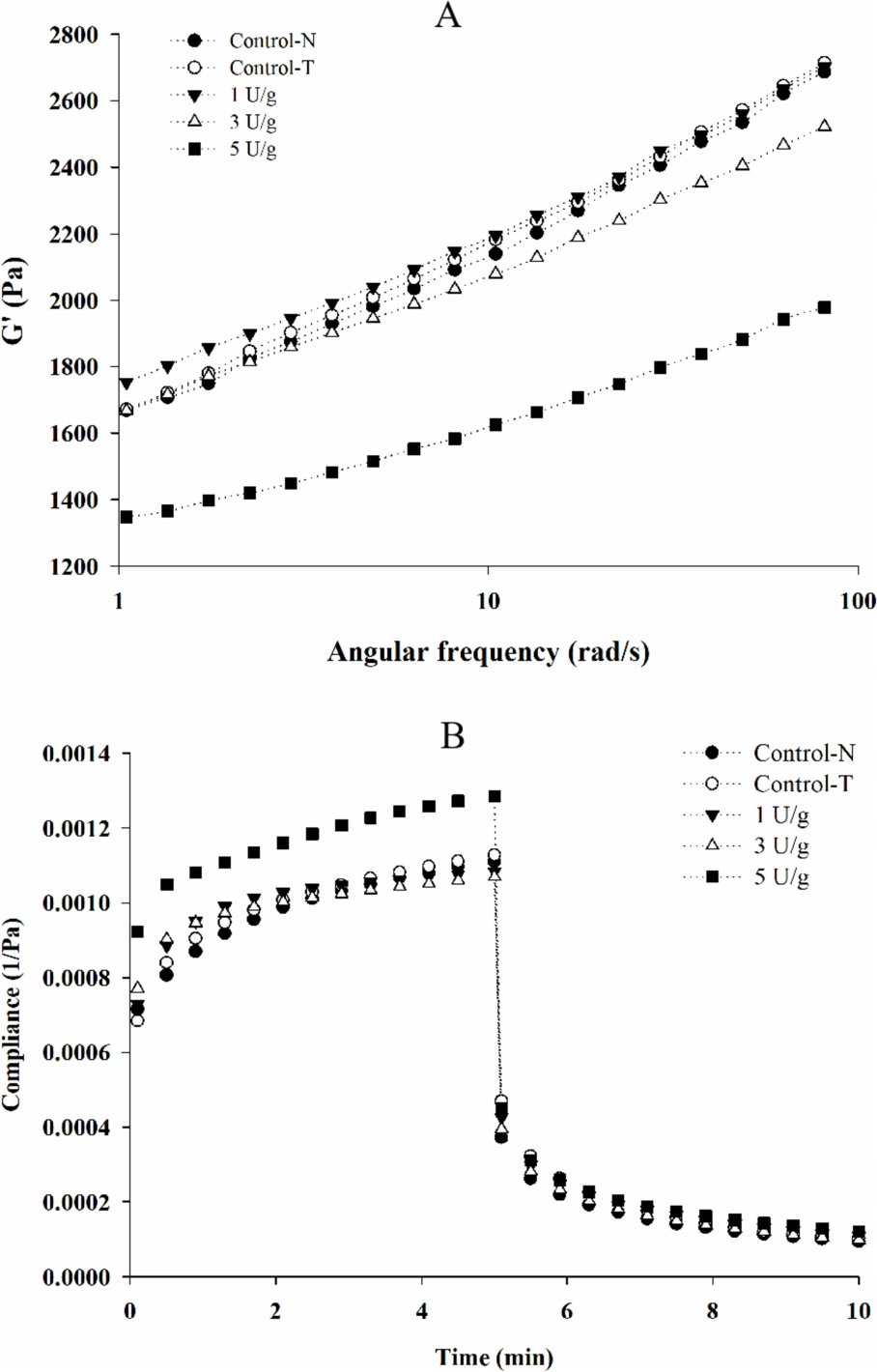
Figure 4. Large deformation of SPI emulsion gels pre-incubated with different concentrations of TGase

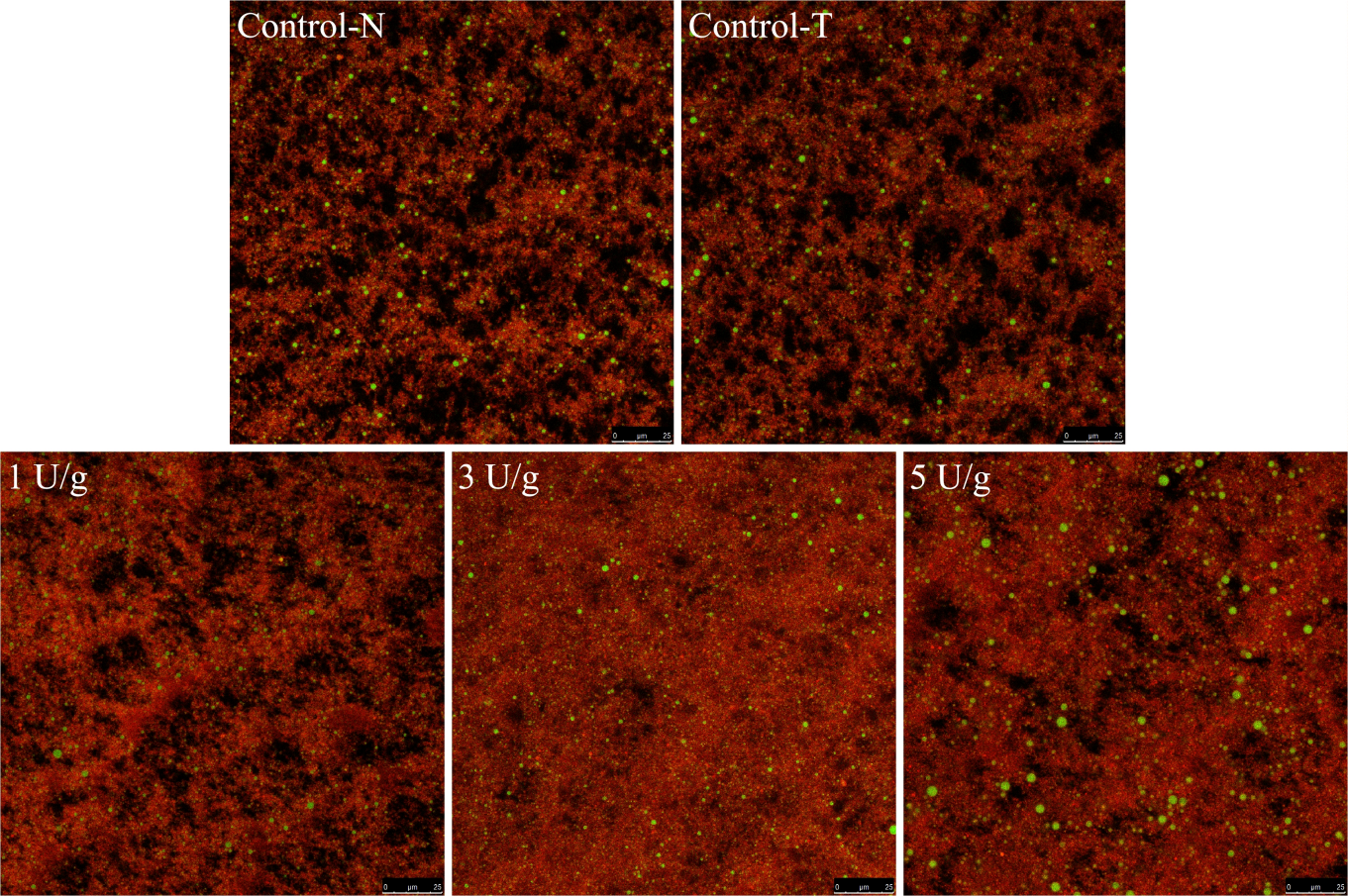
Figure 5. Relative turbidity of the subnatants of the dispersions in various solvents for the SPI emulsion gels pre-incubated with different concentrations of TGase.

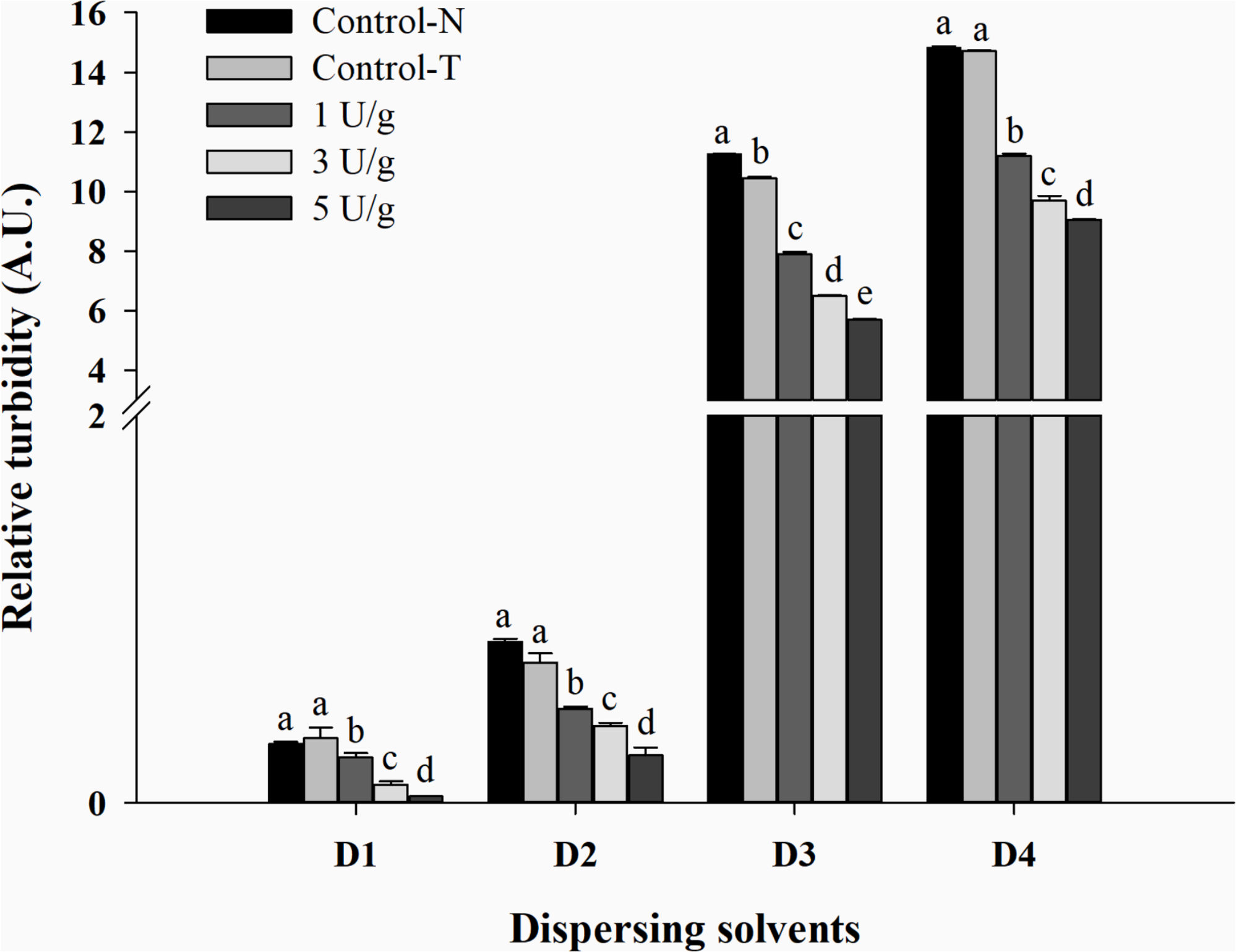
Highlights:

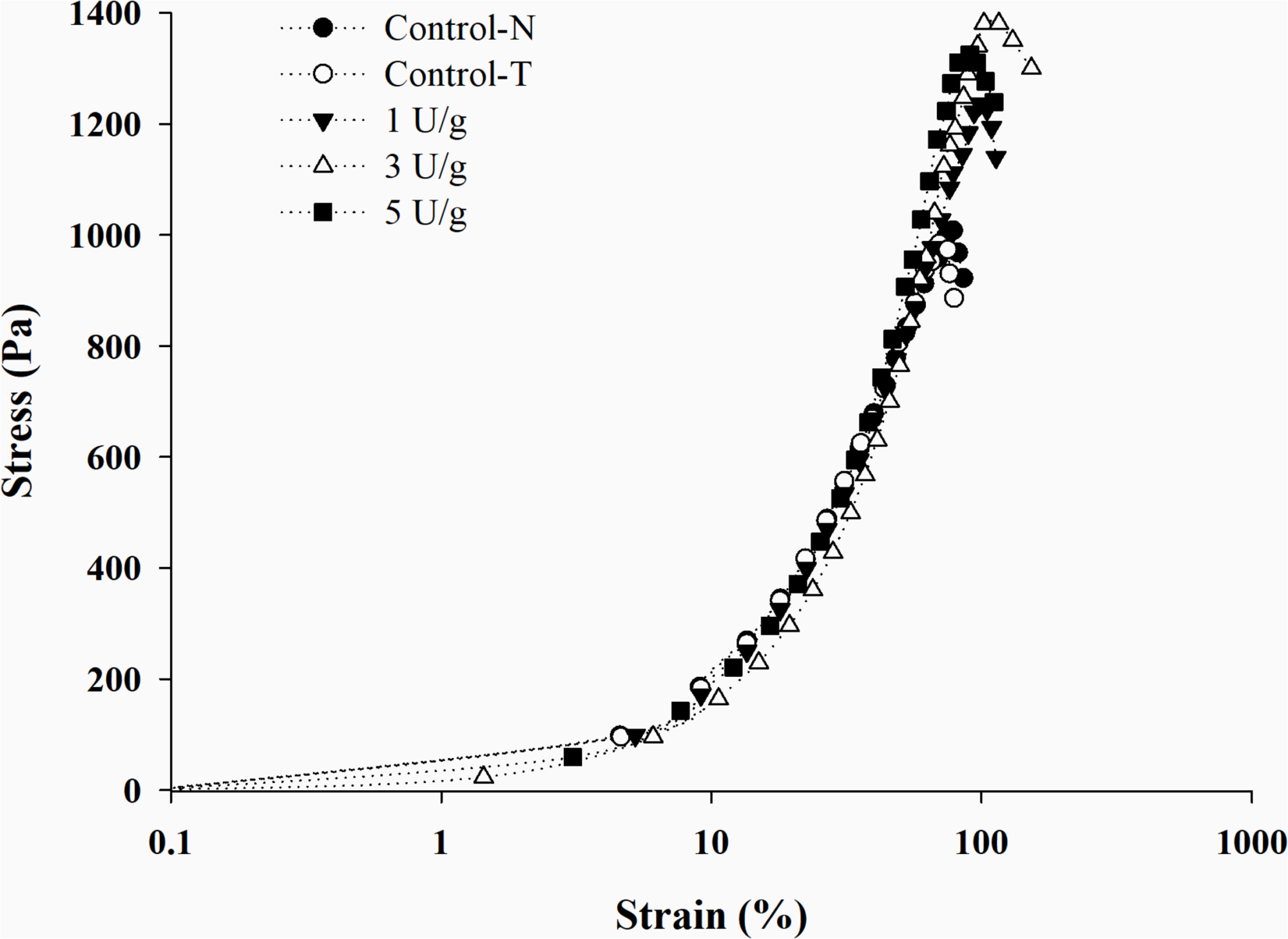
- Effects of TGase pre-crosslinking on the gelation of $CaSO_4$-induced SPI emulsion were investigated.
- Gels pre-incubated with 1-3 U/g TGase improved fracture properties.
- Extensive crosslinking resulted in coarser gel structure with weak firmness.
- Incubation with TGase markedly increased the WHC of SPI emulsion gels.


Control-N
Control-T
1 U/g
3 U/g
5 U/g


A
Control-N
Control-T
1 U/g
3 U/g
5 U/g
G' (Pa)
2800
2600
2400
2200
2000
1800
1600
1400
1200
1
10
100
Angular frequency (rad/s)
B
Control-N
Control-T
1 U/g
3 U/g
5 U/g
Compliance (1/Pa)
0.0014
0.0012
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
0
2
4
6
8
10
Time (min)


Control-N
Control-T
1 U/g
3 U/g
5 U/g


Control-N
Control-T
1 U/g
3 U/g
5 U/g
Relative turbidity (A.U.)
16
14
12
10
8
6
4
2
0
a a b c d
a a b c d
a b c d e
a a b c d
D1
D2
D3
D4
Dispersing solvents


Control-N
Control-T
1 U/g
3 U/g
5 U/g
1400
1200
1000
800
600
400
200
0
Stress (Pa)
0.1
1
10
100
1000
Strain (%)